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A SCANNING ELECTRON MICROSCOPIC STUDY OF EMBRYONIC DEVELOPMENT OF A MARINE HYDROZOAN

VICKI J. MARTIN AND WILLIAM E. ARCHER

Department of Biological Sciences, University of Notre Dame, Notre Dame, Indiana 46556

Abstract

An indepth three-dimensional investigation examined the surface morphological changes associated with the development of a typical marine hydrozoan early cleavage embryo into a mature planula larva. During hydrozoan embryogenesis the arrangement and distribution of surface microvilli change; cilia of some epitheliomuscle cells appear before embryos gastrulate; and the embryo undergoes dramatic changes in body shape. Early cleaving embryos are bizarre in morphology, however, by the end of late cleavage the embryos have rounded to form a sphere. Gastrulation is characterized by the appearance of a blastopore at the future posterior end of the planula and by the migration of cells over the margins of the blastopore to the inside of the embryo. The product of gastrulation is a young planula which elongates and decreases in overall diameter to form a mature planula that eventually attaches via its anterior end to a substrate and undergoes metamorphosis.

INTRODUCTION

The cnidarians display a simple architecture and exhibit exceptional morphogenetic plasticity and adaptability. The phylum is unusual in that its postembryonic development has been much more thoroughly studied than its embryogenesis. Most previous investigations of cnidarian embryogenesis have concentrated on the internal morphology of the planula larva. These studies primarily utilized the techniques of light microscopy and transmission electron microscopy (Martin and Thomas, 1977, 1980, 1981a, b, 1983a; Martin and Chia, 1982; Martin *et al.*, 1983; Walch *et al.*, 1986). Hotchkiss *et al.* (1984) used a scanning electron microscopic cryofracture technique to examine both the surface morphology and the internal morphology of a hydrozoan planula and attempted to correlate their findings with other studies employing transmission electron microscopy. Although the previous work on planulae has contributed valuable information concerning the morphology of the larval form of the cnidarians, major gaps still exist in our basic knowledge of early development in this lower animal phylum. As a result of this lack of information, the following scanning electron microscopic study was undertaken to examine the surface morphological changes associated with the development of a typical marine hydrozoan early cleavage embryo into a mature planula. Such an indepth three-dimensional investigation of the cnidarian embryonic life cycle has never been done. This study provides important information concerning the external morphological changes of embryos during their development and sheds some insight into the morphogenetic shaping of the hydrozoan planula.

Materials and Methods

Mature colonies of the marine hydrozoan *Pennaria tiarella* (McCrady) were collected from pier pilings at the North Carolina Institute of Marine Sciences, Morehead

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City, North Carolina. Fronds from mature male and female colonies were placed together in large finger bowls of filtered seawater. Bowls were placed in the dark at 6:00 p.m., and at 9:00 p.m. they were returned to the light. Embryos in early cleavage stages were observed in the bottoms of the dishes. These embryos were transferred to small dishes of filtered seawater and reared at 23°C to various developmental ages (Table I).

Early cleavage embryos (2–4 hours old), late cleavage embryos (6 hours old), 8-hour embryos (gastrulae), and 10-, 36-, and 48-hour planulae were prepared for scanning electron microscopy (SEM). Animals were fixed for 1 hour in 2.5% glutaraldehyde, pH 7.4, in 0.2 *M* phosphate buffer. They were postfixed for 1 hour in 2% osmium tetroxide in 1.25% sodium bicarbonate, pH 7.2. Samples were dehydrated through a graded series of ethanols and then critical point dried from CO_2 using a Denton critical point dryer equipped with a Tousimis liquid CO_2 water/particulate filter. Animals were coated with gold palladium in a Denton sputter coater. Stubs were examined with a JEOL JSM-T300 SEM operated at 15 kV. Individual cell types were identified according to developmental age of the embryo and surface specializations as previously described in transmission electron microscopic studies (Martin and Thomas, 1977, 1980, 1981a, b). Embryos undergoing gastrulation were continuously examined under a Zeiss light microscope until immature 10-hour planulae were formed.

Results

Cleavage in *Pennaria tiarella* embryos is holoblastic, unequal, and asynchronous (Figs. 1–4) (Martin and Thomas, 1977). The random cleavage pattern results in the formation of blastomeres of unequal size. A period of early cleavage extends from 2 hours postfertilization to the beginning of 6 hours postfertilization. During this time no one embryo cleaves in exactly the same fashion and the embryos exhibit numerous bizarre shapes and reach the 128–256 cell stage (Figs. 1, 2). Early cleavage blastomeres have numerous cytoplasmic blebs extending from their surfaces (Fig. 3). These blebs are transient and disappear in the later cleavage stages. Microvilli arranged in distinct patches project from the surface of each blastomere (Fig. 5). The tips of microvilli in each clump appear to come together at their apical ends to form a point. Early cleavage is very rapid and by 6 to 8 hours postfertilization a solid blastula (late cleavage) is formed (Fig. 6). The blastomeres are more uniform in size than during early cleavage and the embryo assumes the shape of a sphere. The embryo measures *ca.* 230 μm in diameter. During this stage a few cilia appear which are associated with the blastomeres that will give rise to the epitheliomuscle cells of the young planula (Fig. 7). By the end of 6 hours postfertilization the large blebs found in association with the early cleavage blastomeres have disappeared and a mucous-like sheath is seen covering parts of the surface of the late cleaving embryo (Figs. 6, 7). The microvilli of the late cleavage stage are shorter than those seen in earlier stages and are organized into clumps which are not as distinct as in earlier stages (Fig. 8). The microvilli are found on certain regions of the blastomere, while other regions of the same blastomere are devoid of these structures (Fig. 8). Some blastomeres possess few if any microvilli (Fig. 8).

By 8 hours postfertilization the blastomeres are of equal size. The surface of the embryo is smooth, the contours are regular, and a single indentation is present at one pole (Figs. 9, 10). This indentation corresponds to a blastopore, and the pole at which it forms corresponds to the future posterior end of the planula (Fig. 11) (Martin and Thomas, 1983b). Some of the cells at the surface appear to migrate in a radial fashion toward the blastopore, roll over the margins of the pore, and disappear to the inside

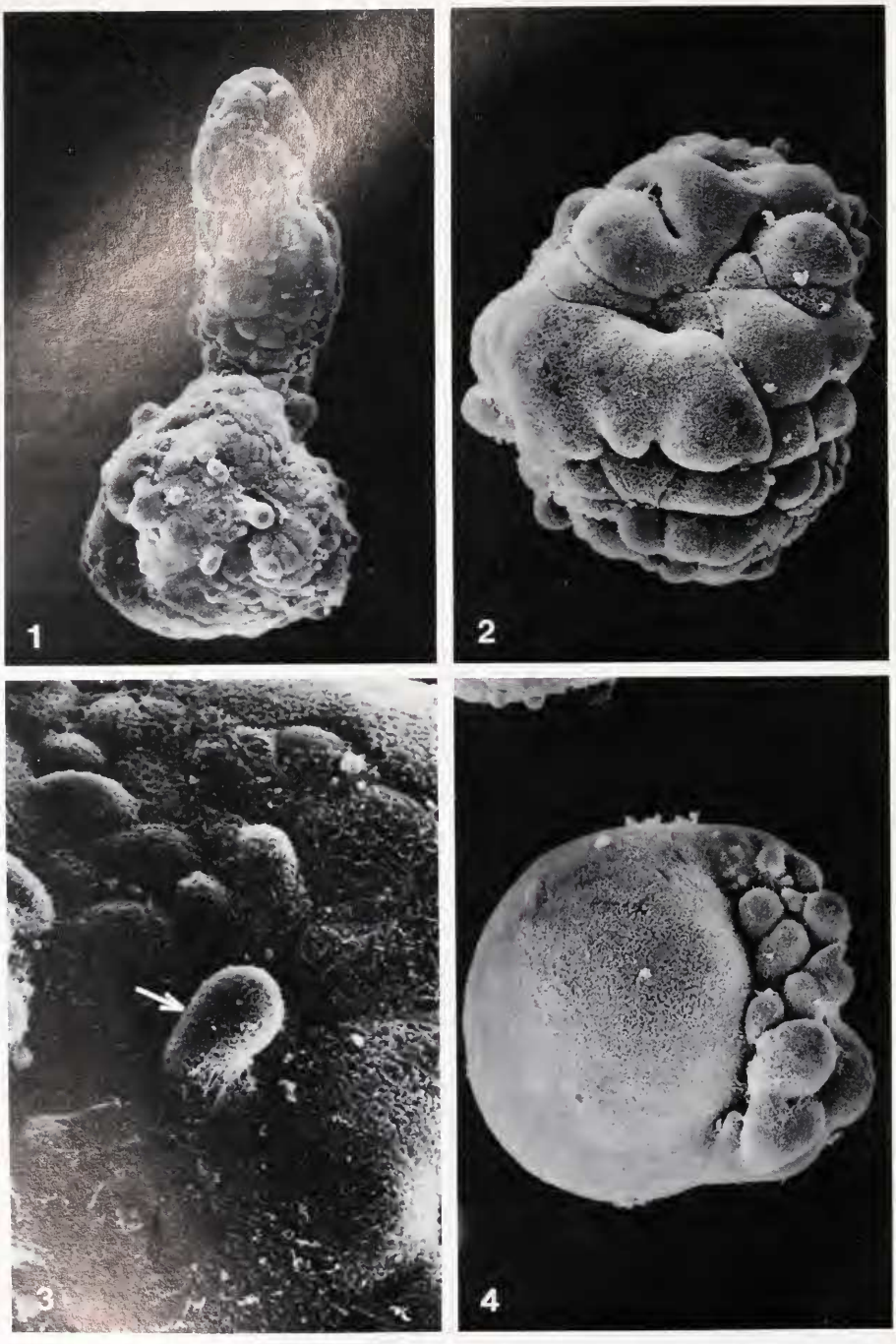
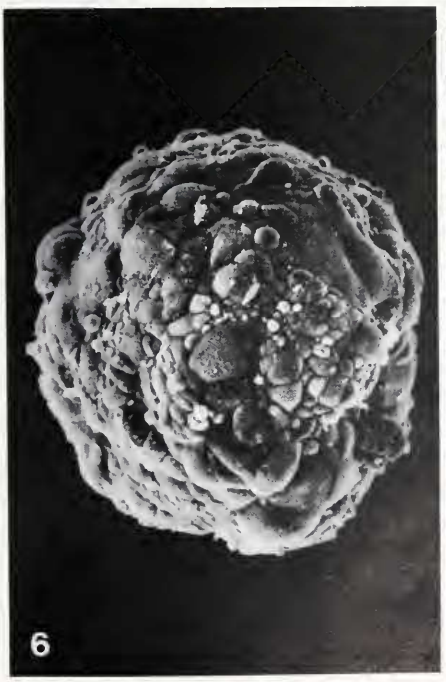


FIGURE 1. Early cleavage embryo (4 hours postfertilization). Cleavage is bizarre and unequal resulting in the formation of small and large blastomeres. ×233.

FIGURE 2. Early cleavage embryo (4 hours postfertilization). The irregular cleavage pattern in this embryo is very different from that in Figure 1. ×290.

FIGURE 3. Early cleavage blastomeres. Numerous cytoplasmic blebs (arrow) project from the early blastomeres. ×1163.

FIGURE 4. Asynchronous early cleavage embryo (2 hours postfertilization). The right side of the embryo is cleaving while the left is not. ×260.



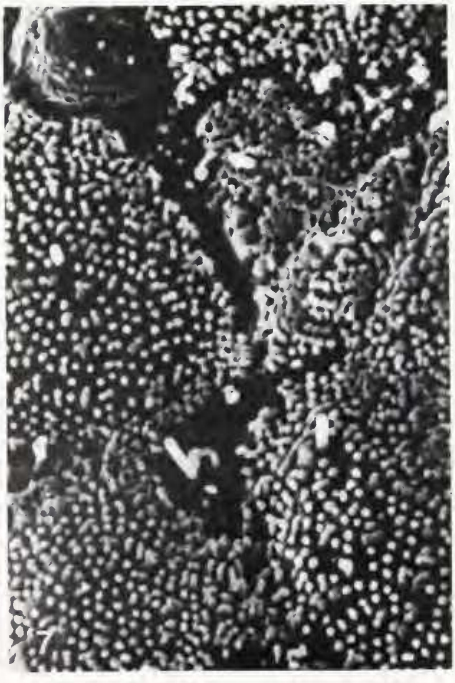


FIGURE 5. Microvilli associated with the blastomeres of an early cleavage embryo (4 hours). Each blastomere is covered with distinct patches of microvilli. The microvilli of each patch converge in their apical regions to form a common focal point. ×5425.

FIGURE 6. Late cleavage (6 hours old). During late cleavage the embryo assumes the shape of a round sphere and the blastomeres are becoming more equal in size. ×290.

FIGURE 7. Late cleavage embryo (just before gastrulation). A few short cilia and mucous appear at the surface before the animal undergoes gastrulation. ×6000.

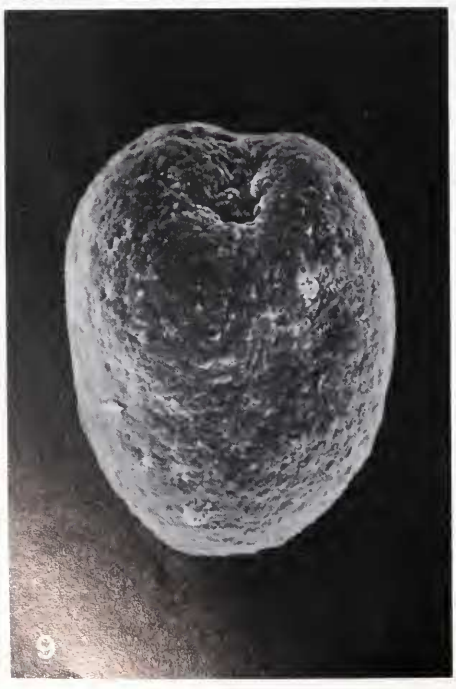


FIGURE 8. Microvilli of the late cleavage embryo (early 8 hours old). Patches of short microvilli cover certain regions of the blastomeres while other areas are bare. The microvilli associated with the patches do not come to a point in their apical regions as seen in early cleavage (Fig. 5). ×7750.

FIGURE 9. Late 8-hour gastrula. The blastomeres are relatively uniform in size and the surface is smooth. An indentation (blastopore) forms at one end of the animal. ×290.

FIGURE 10. Blastopore region. Cells on the outside of the embryo move toward this pore, roll over the margins of the pore, and disappear to the inside. ×1650.

(Martin and Archer, submitted). This stage represents gastrulation. The gastrula is *ca.* 250 μm long and 190 μm wide. During gastrulation surface specializations characteristic of mucous cells appear (see Fig. 16 for gland cell specialization). These specializations consist of a single cilium surrounded by a low disorganized collar of microvilli. The cilium and microvilli sit within a small ectodermal surface depression (Fig. 16). Thus by the end of gastrulation surface projections of mucous cells and epitheliomuscle cells are distinguishable (see Figs. 15, 16). The epitheliomuscle cell possesses a single cilium that projects from its surface. These cilia are short when they first appear and later become longer as the embryo matures to the planula stage (Figs. 7, 15). Very few if any microvilli are associated with this cilium. Each epitheliomuscle cell and mucous cell is monociliated. The surface specializations of the epitheliomuscle cells and mucous cells do not change as the embryo matures, except for the elongation of the cilium. Blastomeres located closest to the blastopore possess short cilia while those farthest from the blastopore have longer cilia. Each blastomere corresponds to either an epitheliomuscle cell or a mucous cell.

Between 8 and 10 hours postfertilization the gastrula elongates in an anterior–posterior direction to form a young planula (Fig. 11). This 10-hour planula measures *ca.* 350 μm long, 180 μm wide in the anterior region, 170 μm wide in the mid area, and 120 μm wide in the tail region. A distinct anterior pole and posterior pole are visible (Fig. 11). The blastopore is located at the posterior pole of the planula and is nearly closed (Fig. 11). The surface cells are numerous, small, and uniform in size. Epitheliomuscle cells and mucous cells comprise the ectoderm. Microvilli of the 10-hour embryo are numerous and are not arranged in distinct patches as seen during early development. They are uniformly distributed over the surfaces of the ectodermal cells. This distribution pattern persists until the planula begins to metamorphose. The 10-hour planula elongates to form the mature planula which is anywhere from 24 to 96 hours old depending upon temperature (Figs. 12, 14). During the elongation period the planula grows in length and becomes narrow in diameter; surface specializations of neurosensory cells and nematocytes appear (Figs. 17, 18); and numbers of cells increase.

By 36 hours the planula is *ca.* 700 μm long, 150 μm wide in the anterior region, 100 μm wide in the mid area, and 80 μm wide in the tail (Fig. 12). The ectoderm of the 36-hour planula consists of epitheliomuscle cells, mucous cells, and nematocytes (see Figs. 15, 16, and 18 for distinguishing surface projections of these cells). The epitheliomuscle cells comprise the majority of the ectodermal cells and are found along the entire length of the planula. Mucous cells are the second major type and are mostly concentrated in the anterior third of the animal (Martin and Archer, submitted). Numerous surface specializations of these cells are abundant in the head region. Nematocytes are located in a region extending from the anterior end of the planula to the lower third of the planula and are most abundant in the anterior end. Apical projections of nematocytes are characterized by a single cilium projecting from a basal collar of long microvilli (Fig. 18). The cilium is displaced to one side of the collar. All cilia at this stage are long (Fig. 13). By 36 hours an indentation is visible at the anterior end of the planula (Fig. 13). This indentation is not the same pore formed by the blastopore during gastrulation. Long cilia project from this indentation. This indentation appears to help the planula attach to a substrate.

At 48 hours the planula is *ca.* 800 μm long, 120 μm wide in the head region, 60 μm wide in the mid area, and 40 μm wide at the tail (Fig. 14). Five types of surface specializations are present by 48 hours (Figs. 15–18). Microvilli are scattered over all the ectodermal cell surfaces. Apical regions of epitheliomuscle cells are characterized by a solitary cilium (Fig. 15). Mucous cells possess a single cilium surrounded by a

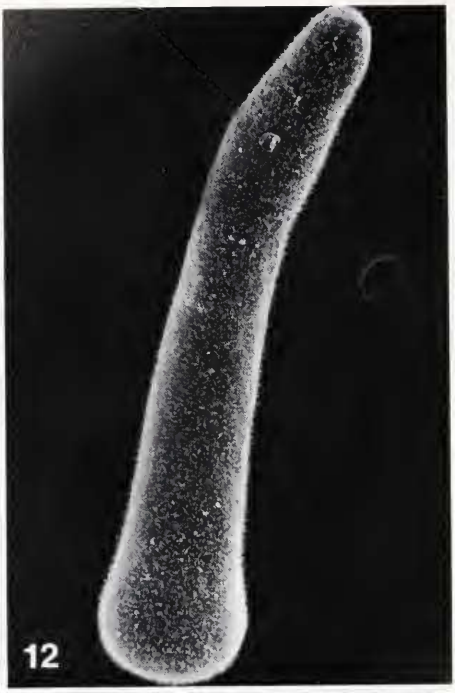


FIGURE 11. Ten-hour planula. The animal has elongated and has a distinct anterior end and posterior end. The anterior end is directed down in this micrograph. Microvilli are scattered over the surfaces of all ectodermal cells. Cilia are numerous. The blastopore (arrow) is located at the posterior tip of the planula. ×280.

FIGURE 12. Thirty-six-hour planula. The planula is long and narrow. Cilia are abundant, long, and scattered over the entire surface of the animal. The anterior and posterior regions are much narrower than seen in the 10-hour planula (Fig. 11). The anterior end is down. ×165.

FIGURE 13. Thirty-six-hour planula. An indentation is present in the middle of the anterior end. This pit is not the same indentation formed by the blastopore. Long cilia project out from the pit. ×1100.

FIGURE 14. Forty-eight-hour planula. Anterior end is directed down. ×145.

loose collar of microvilli, all of which arise from a small surface depression (Fig. 16). Apical extensions of neurosensory cells are visible by this stage (Fig. 17). These sensory cells possess a single cilium surrounded by a bulbous cluster of microvilli. The cilium is located in the center of the cluster. Neurosensory cells occur all along the length of the planula but are most concentrated in the anterior head region of the planula. Nematocyte projections identical to those described for the 36 hour planula are also seen (Fig. 18). The nematocyte specializations are more numerous than those seen at 36 hours, however, they do have the same distribution pattern. Planulae become competent to metamorphose anytime between 24 hours and 96 hours postfertilization depending on temperature. Planulae may metamorphose naturally or they can be induced to metamorphose by treating with cesium chloride (Martin and Archer, in prep.). Shortly after planulae attach to a substrate, their cells lose microvilli and cilia. For a summary of the time sequence of developmental events in embryos of *Pennaria tiarella* at 23°C see Table I.

Discussion

The planula larva is the best described representative of the cnidarian embryonic life cycle (Martin and Thomas, 1977, 1980, 1981a, b, 1983a, b; Freeman, 1981; Martin and Chia, 1982; Martin *et al.*, 1983; Berking, 1984; Walch *et al.*, 1986). The morphological events prior to planula formation have been largely ignored in the past, and as a result information on early development in the cnidarians is lacking (Martin and Thomas, 1977, 1983b; Martin *et al.*, 1983).

In an attempt to understand better embryonic morphogenesis in the cnidarians we used scanning electron microscopy to examine the development of a typical marine hydrozoan beginning with early cleavage and ending with the mature planula. During the development of *Pennaria tiarella* the embryo undergoes extensive changes in body shape. Early cleaving embryos have a bizarre morphology, however, by the end of late cleavage the embryos have rounded to form spheres. Such a rounding of the embryo may be essential if an organized form of gastrulation is to follow. In *Pennaria tiarella* gastrulation is organized with the formation of a blastopore at the future posterior end of the planula and the migration of cells over the margins of the blastopore to the inside of the embryo (Martin and Archer, in prep.). Such movements of cells resemble the morphogenetic process of invagination, a type of gastrulation not previously reported for the Hydrozoa (Tardent, 1978). A similar pattern of gastrulation

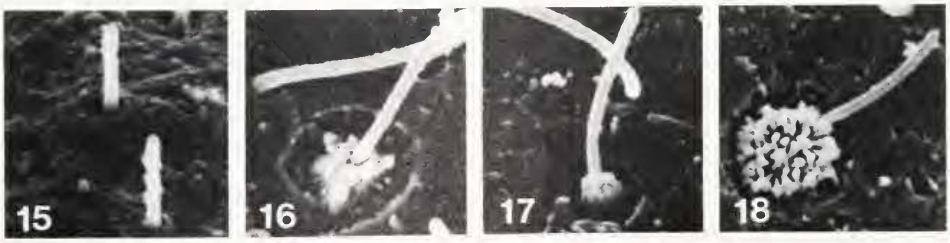


Figure 15. Surface projections of epitheliomuscle cells. A single cilium extends from each cell. ×6500.

Figure 16. Surface projection of a mucous cell. A single cilium surrounded by a disorganized clump of microvilli extends from a slight surface depression. ×6000.

Figure 17. Surface extension of a neurosensory cell. A single cilium projecting from the center of a collar of microvilli characterizes this cell type. ×6000.

Figure 18. Surface extension of a nematocyte. A single cilium projecting from the side of a collar of long microvilli distinguishes the nematocyte. ×6000.

TABLE I

Developmental time table for embryos of Pennaria tiarella

Stage	Developmental age (hours postfertilization)	Distinguishing characteristics
Early cleavage	1–6	Bizarre shape; irregular cleavage; holoblastic cleavage; asynchronous cleavage
Late cleavage	6–8	Holoblastic cleavage; more regular cleavage; more synchronous cleavage; spherical shape; cilia appear
Gastrulation	8–10	Appearance of blastopore; axial elongation; localization of embryonic tissue types; separation of germ layers with the formation of the mesoglea
Early planula	10	Anterior, posterior axis established; closure of blastopore; presence of cilia of epitheliomuscle and mucous cells
Mature planula	24–96	Axial elongation; formation of anterior depression; appearance of surface specializations of neurosensory cells and nematocytes; attachment; metamorphosis

has been observed for embryos of the hydrozoan *Podocoryne carnea,* in which a blastopore is also present (Martin and Archer, submitted). The product of gastrulation is a short fat planula. As the young animal grows into a mature planula capable of attaching and metamorphosing, planular length increases while planular diameter in the anterior, mid, and tail regions decreases.

During development of *Pennaria tiarella* the distribution patterns and numbers of microvilli and cilia change. In early and late cleavage the microvilli are abundant and are found in distinct patches. Cleavages are very rapid in these embryos and the arrangement of microvilli during this period of intense mitotic activity may play an important role in the ability of these blastomeres to adhere together and form stable contacts. The patch arrangement of microvilli may increase the surface area of these projections and hence provide sites for numerous adhesion molecules. As development proceeds through late cleavage cell division slows and the arrangement and number of microvilli change. The number of microvilli per animal increases through the mature planula stage and they are not arranged in patches. The microvilli disappear shortly after the mature planula attaches. At the beginning of gastrulation, the microvilli appear as single entities. Because cell division is slowed, pretty much synchronous, and of a more ordered pattern by gastrulation, perhaps not as many adhesion molecules are needed for the initial sticking together of these cells as were needed earlier. The presence of a single microvilli on the surfaces of cells from early gastrulation through the mature planula stage may be a reflection of the need for fewer adhesion factors during later development.

Cilia are not found on early cleavage embryos. Only after the blastomeres have rounded to form a late cleavage sphere do a few short cilia appear. These cilia are associated with blastomeres that will form differentiated epitheliomuscle cells after gastrulation. The presence of cilia on these late cleavage blastomeres may indicate that some of these early cells are predetermined during cleavage before the actual separation of the two germ layers occurs during gastrulation. As development continues to the mature planula stage cilia grow in length and increase in number. Surface specializations characteristic of mucous cells, neurosensory cells, and nematocytes form. The appearance of these specializations at particular developmental ages as

determined from scanning electron microscopy corresponds well to their developmental appearance noted using transmission electron microscopy (Martin and Thomas, 1977, 1980, 1981b, 1983a).

Sexual reproduction and embryogenesis have never been a primary focal point in cnidarian research. This is surprising because the cnidaria offer excellent material for the study of the evolution of embryogenesis. Embryogenesis in the simpler forms at times appears almost "anarchic," whereas, in the more advanced cnidaria highly complex mosaic patterns are seen (Metschnikoff, 1886; Carré, 1969). The present study examines embryogenesis in a marine hydrozoan. Findings such as the arrangement and distribution of microvilli during development, the presence of cytoplasmic blebs on early blastomeres, the presence of cilia during late cleavage, the formation of a blastopore, the migration and invagination of surface cells during gastrulation, and the changes in body shape as the hydrozoan embryo progresses from early cleavage to the mature planula have not been reported previously. Additional studies of embryogenesis in the phylum are needed to fill in the major gaps that exist in our basic knowledge of morphogenesis in this lower animal phylum.

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